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Microbial-Derived Butyrate: An Oncometabolite or Tumor-Suppressive Metabolite?

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Dietary factors, microbial composition, and metabolism are intimately intertwined into a complex network whose activities influence important intestinal functions. In a recent issue of *Cell*, Belcheva et al. (2014) show that microbial-derived butyrate promotes proliferation of cancer-initiated intestinal epithelial cells, suggesting that it can act as an oncometabolite.

Colorectal cancer (CRC) is a complex pathology integrating various host genetic components, nutrition, inflammation, and most recently, microbial composition and associated activities. The latter has gained attention for both cancer-promoting as well as cancer-protecting effects. The events dictating these microbial-driven contradictory outcomes are not clear, and intense investigations are directed toward elucidating them. Since the inception more than 5 years ago of large-scale microbiome studies funded by various worldwide agencies, the scientific community has compiled an impressive catalog of microbial communities living at several body sites during various phases of life and under healthy versus pathological conditions. With a large majority of microorganisms residing in the colon, it comes as no surprise that this location has been under intense scrutiny regarding the relationship between microbes and pathologies such as necrotizing enterocolitis, inflammatory bowel diseases (pediatric and adult), recurrent *Clostridium difficile* infections, irritable bowel syndrome, and CRC. Although these studies have identified differences in microbial compositions between healthy individuals and those afflicted by various disease states, knowledge of the functional consequences of these microbially unbalanced conditions is more limited. For example, numerous studies in patients with CRC have established the presence of bacterial dysbiosis, but the functional impact of the microbial imbalances is still unknown.

Although efforts are still directed at identifying microbes associated with CRC, microbial functions have gained tremendous attention as plausible causative factors of carcinogenesis. As a whole, the microbiome has a prodigious metabolic capacity, including the production of diverse bioactive food components and micronutrients such as essential vitamins, and the fermentation of dietary fibers and complex carbohydrates into short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate. Diet is recognized as a key environmental factor for cancer susceptibility, yet there is limited insight regarding the interplay between nutrition, microbes, and carcinogenesis. In a recent issue of *Cell*, Belcheva et al. demonstrate that either antibiotic treatment or a low-carbohydrate diet attenuated intestinal polyp formation (a precursor to CRC) in a mouse model by decreasing bacterial-derived butyrate and diminishing epithelial cell proliferation (Belcheva et al., 2014). The study shows how diet and microbial activities contribute to cancer development and highlights the need to decipher the interplay between host genetics, microbes, nutrition, and carcinogenesis.

To assess the impact of the microbiota on carcinogenesis, Belcheva et al. (2014) utilized an established model of human adenomatous polyposis, the $Apc^{Min/+}$ (multiple intestinal neoplasia) mice, that were also deficient for the DNA mismatch repair gene *MutS homolog 2* (*Msh2*). These $Apc^{Min/+}$;$Msh2^{-/-}$ mice showed enhanced tumor number, particularly in the colon, compared to $Apc^{Min/+}$;$Msh2^{+/-}$ mice. When $Apc^{Min/+}$;$Msh2^{-/-}$ mice were exposed to broad-spectrum antibiotic treatment, they displayed a strong reduction in polyp formation in both the small intestine and the colon at 6 weeks of age. Interestingly, 16S phylogenic analysis showed that the microbiota of untreated $Apc^{Min/+}$;$Msh2^{-/-}$ mice and $Apc^{Min/+}$;$Msh2^{+/-}$ mice were similar, suggesting that microbial activities, rather than selective microbial species differences, were responsible for the enhanced tumor burden. Host inflammatory environment and/or epithelial DNA damage have been identified as cancer-promoting events, with some of these factors being influenced by microorganisms (Schwabe and Jobin, 2013). However, Belcheva et al. did not observe any differences in the distribution of immune cells between $Apc^{Min/+}$;$Msh2^{-/-}$ mice and $Apc^{Min/+}$;$Msh2^{+/-}$ mice or modulation of cancer development following genetic manipulation of immune genes ($Rag1^{-/-}$, caspase $1^{-/-}$). Similarly, somatic mutations or γH2AX prevalence was not different between genotypes or after antibiotic treatment, suggesting that cancer promotion in $Apc^{Min/+}$;$Msh2^{-/-}$ mice operates through a different mechanism(s). Indeed, expression of the proliferative markers Ki-67 and β-catenin was enhanced, while the differentiation marker p21 (Cip1/WAF1) was reduced in $Apc^{Min/+}$;$Msh2^{-/-}$ mice.

The ability of the microbiota to biotransform various dietary components into functional metabolites is likely incapacitated when the ecosystem is disrupted by antibiotic treatments or altered by dietary manipulation. Belcheva et al. (2014) tested the effect of a low-carbohydrate diet on microbial community composition and cancer development in $Apc^{Min/+}$;$Msh2^{-/-}$ mice. While the low-carbohydrate diet had no modulatory effect on microbial community composition

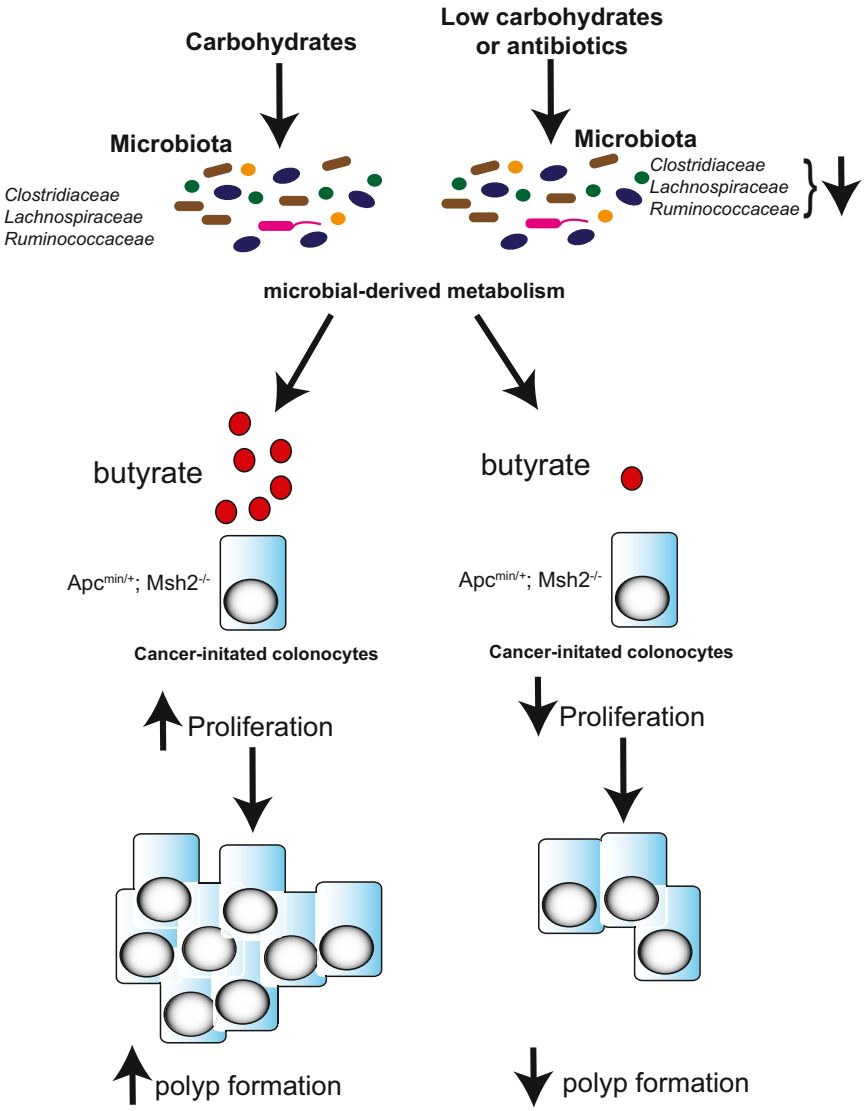

Figure 1. Microbiota-Derived Metabolism of Carbohydrates Promotes Polyp Frequency in the Intestine

Butyrate-producing bacteria such as *Clostridiaceae*, *Lachnospiraceae*, and *Ruminococcaceae* biotransform carbohydrates into butyrate, which, in the context of cancer-initiated colonocytes such as those observed in the epithelium of $Apc^{Min/+}$;$Msh2^{-/-}$ mice, foster cellular proliferation and polyp formation. The microbiota of $Apc^{Min/+}$;$Msh2^{-/-}$ mice exposed to low-carbohydrate diet or antibiotics is impaired in its metabolic capacitiy and butyrate formation, thereby preventing cancer-initiated colonocyte proliferation and cancer progression.

or body weight, the number of polyps was reduced in both the small bowel (~2-fold) and the colon (~6-fold) to a level similar to antibiotic treatment. Abundance of SCFA-producing bacteria *Clostridiaceae*, *Lachnospiraceae*, and *Ruminococcaceae* was reduced by antibiotic treatment or a low-carbohydrate diet. SCFAs such as acetate and butyrate have profound effects on intestinal mucosal biology, affecting both epithelial cell proliferation and mucosal immune response. Interestingly, liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis showed that butyrate was significantly diminished following either cocktail antibiotic treatment or a low-carbohydrate diet, both being conditions that attenuated polyp number in $Apc^{Min/+}$;$Msh2^{-/-}$ mice. To functionally determine the role of butyrate in polyp formation, this particular SCFA was directly introduced either by diet (tributyrin) or sodium butyrate enemas, which mostly target the small intestine and colon, respectively. Strikingly, these butyrate supplementation strategies reversed polyp reduction afforded by antibiotic treatment in $Apc^{Min/+}$;$Msh2^{-/-}$ mice and promoted epithelial cell proliferation and tumor progression. These findings suggest that microbial-derived metabolism of carbohydrates into SCFA such as butyrate may fuel proliferation of cancer-initiated epithelial cells, thereby promoting carcinogenesis (Figure 1).

Interestingly, the findings of Belcheva et al. suggest that butyrate functions as an oncometabolite, a provocative thought since numerous previous studies have identified butyrate as a tumor-suppressive metabolite (Bultman, 2014). In addition, ≥5 human microbiome sequencing projects have reported that CRC cases have decreased abundance of butyrate-producing bacteria compared to healthy controls. Furthermore, when butyrate is added to CRC cell lines, it decreases cell proliferation while increasing apoptosis and/or cell differentiation. In fact, butyrate-induced expression of p21 is responsible for the decreased proliferation of HCT116 cells (Archer et al., 1998). Yet, in the $Apc^{Min/+}$;$Msh2^{-/-}$ model, Belcheva et al. observed increased butyrate levels in untreated mice were correlated with decreased expression of p21 (and increased cell proliferation and polyp number) compared to mice treated with antibiotics or provided a low-carbohydrate diet. How does one reconcile these seemingly disparate findings? Butyrate is a pleiotropic molecule that functions as an energy source, a histone deacetylase (HDAC) inhibitor, and an agonist of several G protein-coupled receptors. It may thus have different effects depending on the genetic background of the host. In this regard, $Apc^{Min/+}$;$Msh2^{-/-}$ tumor initiation does not necessarily involve dysregulated β-catenin expression, which is different from the more commonly studied $Apc^{Min/+}$ and azoxymethane (AOM) models (Kongkaanuntn et al., 1999). Furthermore, MSH2 deficiency results in a mutator phenotype that magnifies the somatic genetic background differences in the tumor (Reitmair et al., 1997). In addition to genetic background, age is a possible confounding element because Belcheva et al. analyzed their $Apc^{Min/+}$;$Msh2^{-/-}$ mice at 3–6 weeks of age, whereas most

other mouse models of CRC are analyzed at adult stages (>8 weeks). It would be important to confirm these findings using different CRC models (e.g., *Smad3*$^{-/-}$) and also to determine whether the deleterious effect of butyrate is mediated through G protein-coupled receptors (Jobin, 2014). For example, reducing butyrate levels through dietary manipulation (fiber) or molecular deletion (*Gpr109a*$^{-/-}$) augmented polyp formation in *Apc*$^{Min/+}$ mice, a phenomenon linked to decreased T regulatory (Treg) cell differentiation (Singh et al., 2014).

It is worth noting that this is not the first instance of a "butyrate paradox." Butyrate has long been known to have differential effects on normal versus cancerous colonocytes, and only recently has this been addressed. Due to the Warburg effect, butyrate is metabolized by cancerous colonocytes to a lesser extent and therefore accumulates as an HDAC inhibitor (Donohoe et al., 2012). Similarly, butyrate may have heterogeneous effects on tumorigenesis depending on host genetic background, the presence of other bacterial metabolites such as an omega-3 fatty acid (docosahexaenoic acid), which synergizes with butyrate to induce colonocyte apoptosis (Kolar et al., 2007), and whether it is exerting a direct effect on the tumor (cell autonomous) versus non-cell-autonomous effects such as regulating mucosal immune cell activity as mentioned above. Therefore, although the current study contributes to our understanding of the interplay between diet, microbes, and CRC, the role of butyrate in cancer protection/promotion will still require further investigation. Altering microbial activities through dietary manipulation represents an exciting means to harness the microbiome and influence health and disease states. Whether dietary manipulation could be used effectively to preserve homeostatic functions afforded by microbiota while attenuating its potential pathological effects is still an open question, and more research would be necessary before this strategy becomes a reality.

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What Lies Within: Coinfections and Immunity

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Helminth-induced immunomodulation is thought to influence the outcome of secondary infections. Osborne et al. (2014) and Reese et al. (2014) demonstrate that helminth infection impacts viral infections by tilting the immune system toward Th2/M2 immune regulatory responses that dampen Th1/M1 antiviral responses as well as promote reactivation of latent herpesviruses.

The mammalian intestine is home to many pathogens, including commensal bacteria, helminth parasites, and viruses. Among these, helminths represent some of the earliest recorded human infections in history and remain a significant source of infection today. Approximately 2.7 billion people who live in low-income countries in Africa, South America, and Asia are thought to have some type of helminth infection (Hotez et al., 2007). In addition to infectious complications, helminths are also associated with human malignancy. *S. hematobium* is a platyhelminth that is associated with bladder cancer, particularly in Egypt. Additionally, *O. viverrini* (liver fluke) and *C. sinesis* are classified as group 1 carcinogens by the International Agency for Research on Cancer (IARC) (Bouvard et al., 2009) and are causally associated with cholangiocarcinoma, which is highly prevalent throughout much of southeast Asia and Egypt. The presumed mechanisms include chronic inflammation and hyperplasia of billary epithelium.

Helminth-induced immunomodulation has long been thought to influence human